hydrophobic group-containing polysaccharides formed by the present invention, each aggregate is formed from 3-20 molecules of the hydrophobic group-containing polysaccharides under association of the molecules with each other by coupling together the hydrophobic groups and the molecules of the polysaccharide mainly through hydrophobic coupling. The "number of associations" means the number of molecules found under this molecular association.

Claims 1-6 have been rejected under 35 USC 103(a) as being unpatentable over Shiku et al or Macromolecules in view of Okumura et al. Applicants respectfully traverse this ground of rejection and urge reconsideration in light of the following comments.

As discussed in the present specification, hydrophobic group-containing high molecular weight polysaccharides have been used in pharmaceutical applications as a coating material for coating a drug carrier having a drug enclosed therein. These types of polysaccharides have been favorably used due to their suppression of spontaneous exudation of the drug from the drug carrier and their improvement in the cell-specific drug transference rate. Conventionally, the hydrophobic group-containing polysaccharides have been formed into aggregates by two different processes. The first process requires a dissolving of the hydrophobic group-containing polysaccharide in dimethyl sulfoxide under a dilute condition and then dialyzing the resulting solution against water. The second process involved causing the hydrophobic group-containing polysaccharide to swell in water and then treating the resulting swollen dispersion by ultrasonication.

These prior art processes have problems in that it is difficult to prepare the aggregates of the hydrophobic group-containing polysaccharide industrially in large amounts. In the dialysis process, a large dialysis equipment arrangement is necessary in order to perform large-scale treatment, a huge amount of water is required and a prolonged period of time is

required for treatment. In the ultrasonication process, problems arise in that the through-put of one single treatment is lowered, the deviation between the products is large since control of the sonication efficiency and sonication time is difficult and monodispersed aggregates are not able to be obtained steadily and there is a possibility of contamination with fractured metal fragments due to deterioration of the sonication tip. The present invention overcomes the problems associated with the prior art and provides a process for forming aggregates of a hydrophobic group-containing polysaccharide in which the deviation between the products and contamination by impurities are eliminated and enables the preparation of uniform aggregates of the hydrophobic group-containing polysaccharide steadily in a simple and convenient manner within a brief period of time and on a large-scale.

The presently claimed invention is directed to a process for forming aggregates of a hydrophobic group-containing polysaccharide comprising the steps of mixing the hydrophobic group-containing polysaccharide with water in an amount of 30 - 10,000 times the weight of the hydrophobic group-containing polysaccharide to form a swollen dispersion of the hydrophobic group-containing polysaccharide and treating the swollen dispersion of the hydrophobic group-containing polysaccharide with a homogenizer under a pressure of from 9.8 - 490 MPa to form a dispersion of monodisperse aggregates of the hydrophobic group-containing polysaccharide.

In the process for forming aggregates of a hydrophobic group-containing polysaccharide according to the present invention, the starting raw material, i.e. the hydrophobic group-containing polysaccharide, is typically provided in the form of a powder. When water is added thereto, individual particles will become swollen with water and the swollen particles will associate together to form jelly-like aggregated lumps suspended in water. By processing the resulting suspension of the jelly-like lumps by homogenizing

it using a high pressure homogenizer, the aggregated swollen lumps are finely disintegrated into a dispersion, in which some 3 - 20 molecules of the polysaccharide become associated with each other to form a stable monodisperse dispersion of relatively small aggregates.

In the resulting monodisperse dispersion of the polysaccharide, relatively small aggregates of about 3 to 20 molecules are formed by coupling together the hydrophobic groups intra- or intermolecularly during the course of being dispersed by the high pressure homogenizer. Therefore, the monodisperse dispersion of the aggregates is spontaneously formed during the homogenization treatment by the homogenizer without necessitating special process step of forming the aggregates by molecular association. The technical term "monodisperse" means a state of uniform dispersion in which the size and the configuration of each particle of the dispersed phase are highly uniform.

According to the present invention, the causing of the hydrophobic group-containing polysaccharide to swell in water constitutes an essential feature for obtaining a monodisperse aggregate dispersion. In the process according to the present invention, a hydrophobic group-containing polysaccharide is caused to swell with water by admixing water with the starting hydrophobic group-containing polysaccharide in a weight proportion of 30 - 10,000 times the weight of the polysaccharide. As discussed in the original English specification on page 14, lines 12 - 17, use of water in an amount below 30 times the weight of the hydrophobic group-containing polysaccharide may result in formation of an unsuitable gelled product, whereas use of water in an amount exceeding over 10,000 times the weight of the polysaccharide will cause a decrease in the efficiency of the aggregate formation.

The present invention exhibiting the inventive features as given provides a simple but novel process for forming

aggregates of a hydrophobic group-containing polysaccharide useful as a coating material of a drug carrier. It is respectfully submitted that the presently claimed invention is patentably distinguishable over the prior art cited by the Examiner.

In the outstanding Office Action, the Examiner states that the currently presented claims differ from the process of the Shiku et al patent and the Macromolecules reference in that the present invention requires the step of dispersing the swollen dispersion be performed with a homogenizer as opposed to treatment by ultrasonication. A copy of the Macromolecules reference is enclosed herewith as per the Examiner's request. In order to show the interchangeability of a homogenizer with an ultrasonicator, the Examiner has cited the Okumura et al reference as a secondary reference.

The Okumura et al reference is directed to a silver halide photographic light-sensitive material comprising a support and a silver halide emulsion layer provided on the support. The silver halide emulsion layer comprising a hydrogen peroxide-treated gelatin in a ratio of not lower than 20% by weight to the total amount of gelatin contained in the silver halide emulsion layer and silver halide grains composed of silver chlorobromide having a silver chloride content of not lower than 90 mol %. While this reference does suggest the interchangeability of a homogenizer and an ultrasonicator in the emulsification in the dye-forming couplers and other hydrophobic components which are dissolved in a high boiling solvent having a boiling point of over 150°C in the presence of a low boiling organic solvent and/or water-soluble organic solvent, which is then emulsified in a hydrophobic binder such as an aqueous solution of gelatin, this reference has no teachings that would suggest to one of ordinary skill in the art that there is any equivalence between a homogenizer and an ultrasonicator in the dispersions disclosed in the primary references. The viscosities and make-ups of the dispersions

of the present invention, and those of the primary references, are completely different from the emulsions formed in Okumura et al. A showing of equivalence between an ultrasonicator and a homogenizer in the process of Okumura et al is not sufficient to establish an equivalence between these two pieces of equipment in all other applications. Therefore, Applicants respectfully submit that only hindsight provided by the instant disclosure is providing the motivation to combine the references in the manner suggested by the Examiner. Moreover, even if the motivation of the references existed to make the combination suggested by the Examiner, the presently claimed invention still would be patentably distinguishable over the prior art cited by the Examiner.

The process according to the present invention is essentially different from the combined teachings of Macromolecules, Shiku et al and Okumura et al in that the resulting product of the present invention is a monodisperse aggregate dispersion, as compared to the combined teachings of the references which do not disclose a monodisperse dispersion.

Secondly, use of a homogenizer according to the present invention is required to attain a monodisperse aggregate dispersion, whereas the ultrasonication, disclosed in Macromolecules, is not able to provide a monodisperse dispersion as shown by a comparison of the results of Example 1-1 (Fig. 1(b)) and of Comparative Example 2 (Fig. 5) in the present invention. In Example 1-1, the dispersing treatment is effected by using a high pressure homogenizer, whereas, in Comparative Example 2, the dispersing treatment is performed by ultrasonication. From Fig. 1, it is seen that a monodisperse aggregate dispersion is formed in Example 1-1, whereas it is seen from Fig. 5 that a shoulder remains at the peak for Comparative Example 2, even after 60 minutes' ultrasonication, which indicates that the resulting aggregates are not monodisperse as compared with that of Example 1-1.

As shown in Comparative Example 2 of the present application, a dispersion of monodisperse aggregates cannot be attained by effecting the ultrasonication after the hydrophobic group-containing polysaccharide has been subjected to a swelling treatment with water, as contrasted to the process according to the present invention. This means that a monodisperse aggregate dispersion is not obtained by simply replacing the homogenizer with an ultrasonication means. Therefore, a person of ordinary skill in the art would expect only the same effect as that by ultrasonication, even if treatment using a homogenizer is disclosed in Okumura et al, since a homogenizer is disclosed as a dispersing means equivalent to ultrasonication.

According to the present invention, a monodisperse aggregate dispersion can be attained not only by the use of a homogenizer but also through the inventive features of performing the process under the claimed condition of swelling under a specific pressure, so that the present invention is distinguishable over the cited references.

Reconsideration of the present application and the passing of it to issue is respectfully solicited.

Respectfully submitted,

TFC/smd

Terryence F. Chapman

FLYNN, THIEL, BOUTELL & TANIS, P.C.
2026 Rambling Road
Kalamazoo, MI 49008-1699
Phone: (269) 381-1156
Fax: (269) 381-5465

Dale H. Thiel	Reg. No. 24 323
David G. Boutell	Reg. No. 25 072
Ronald J. Tanis	Reg. No. 22 724
Terryence F. Chapman	Reg. No. 32 549
Mark L. Maki	Reg. No. 36 589
David S. Goldenberg	Reg. No. 31 257
Sidney B. Williams, Jr.	Reg. No. 24 949
Liane L. Churney	Reg. No. 40 694
Brian R. Tumm	Reg. No. 36 328
Tricia R. Cobb	Reg. No. 44 621

Encl: Macromolecules Article
Marked-Up Amended Specification and Abstract
Substitute Abstract
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IN THE SPECIFICATION

Please amend the specification as follows.

Please replace the paragraphs beginning on page 1, line 1, and ending on page 6, line 20, with the following rewritten paragraphs.

FIELD OF THE TECHNIQUE

The present invention relates to a process for forming aggregates (associated products) of hydrophobic group-containing polysaccharides.

BACKGROUND OF THE TECHNIQUE

Hydrophobic group-containing high molecular weight polysaccharides in which hydrophobic group(s) are bound to a polysaccharide are used for medicinal materials, for example, coating material for coating a drug carrier enclosing therein a drug. It is known that, by coating a drug carrier, for example, a liposome microcapsule, microsphere, O/W emulsion or erythrocyte ghost, with a hydrophobic group-containing polysaccharide, not only the spontaneous exudation of a drug from such a drug carrier is suppressed but also the cell-specific drug transference rate using such a drug carrier is improved.

It has in recent years been widely accepted that liposomes and O/W emulsions are prospective as drug carriers. It has been reported that the chemical and physical stabilities of a drug carrier of this kind within and without a living body are improved by coating the drug carrier with a polysaccharide, wherein thereby a target-tropism to a specific cell group is also revealed {Bull. Chem. Soc. Japan, 62, 791-796 (1989)}. It has further been reported that liposomes are physically

stabilized by coating them with a polysaccharide {Drug Delivery System, 5, 261 (1990)}.

Further, it is reported that hydrophobic group-containing polysaccharides interact with proteins and with compounds exhibiting higher hydrophobicity so as to encapsulate these proteins or compounds {Chem. Lett., 1263 (1991)}. In this literature, it is described that, when aggregates of a hydrophobic group-containing polysaccharide are mixed with a globular protein of varying kinds at room temperature, the protein becomes coupled with the aggregates of the hydrophobic group-containing polysaccharide to form a conjugate. Therein is also described that aggregates of hydrophobic group-containing polysaccharides are stable, even in the presence of excess amounts of such proteins.

Further, a vaccine product containing a hydrophobic group-containing polysaccharide and an antigen is also known (WO 98/09650). It is furthermore known that a conjugate of a hydrophobic group-containing polysaccharide and an antigen can be isolated and purified by mixing aggregates of the hydrophobic group-containing polysaccharide with the antigen at room temperature, and, then, treating the resulting mixture by gel chromatography {Macromolecules,. 7654 (1994)}.

On the other hand, Akiyoshi et al disclose in Macromolecules,. 3062 (1993) that ~~a~~hydrophobicized polymeric substances are subject to intra- or inter-molecular self association of their hydrophobic groups in a dilute aqueous solution, resulting in formation of aggregates of the polymer molecules. In particular, a hydrophobic group-containing polysaccharide forms relatively monodisperse microparticles of aggregates in a size of nano-order in a dilute aqueous solution by

spontaneous association of several molecules. It is confirmed by observation under electron microscope that relatively monodisperse globular microparticles of nano-order size are formed. Such relatively mono-disperse nano-order size aggregates of the hydrophobic group-containing polysaccharide exist in water as a dispersion which is colorless and transparent in appearance and does not form any cloud or precipitate, even after allowing to stand~~ing still~~ for a long period of time, leaving an appearance of an aqueous solution in the human eye.

By causing a hydrophobic group-containing polysaccharide to swell in water and agitating the resulting swollen dispersion using, for example, a homomixer, a turbid dispersion is obtained. In such a turbid dispersion, a part of the hydrophobic group-containing polysaccharide forms aggregates of a size of nano-order, while there are at the same time some which are present as lumps of various sizes without forming such aggregates. When a turbid liquid, in which lumps of sizes greater than nano-order size are present, is used for a medicinal material, for example, as a material in a drug delivery system (DDS) for intravenous administration, thrombus may be formed due to the above-mentioned lumps. When, on the other hand, the colorless transparent liquid in which the hydrophobic group-containing polysaccharide is present, forming aggregates of uniform nano-order size, is used therefor, there is no fear of thrombus formation. Therefore, there is a demand for aggregates of a hydrophobic group-containing polysaccharide which are dissolved (dispersed in a colorless transparent state) in water, in order to use the hydrophobic group-containing polysaccharide as, for

example, a medicinal material for building up a conjugate with a varying kind of drug or protein.

In the past, processes have been known for forming hydrophobic group-containing polysaccharides into aggregates, for example, 1) a process in which the hydrophobic group-containing polysaccharide is dissolved in dimethyl sulfoxide (DMSO) under a dilute condition and the resulting solution is then dialyzed against water and 2) a process in which the hydrophobic group-containing polysaccharide is caused to swell in water and the resulting swollen dispersion is then treated by ultrasonication {Macromolecules,. 3062 (1993); WO 98/09650}.

It is, however, quite difficult to prepare such aggregates of a hydrophobic group-containing polysaccharide industrially in large scale by the above-mentioned processes of the prior art. Firstly, for example, in the dialysis process of the prior art, there are problems in that 1) a dialysis arrangement capable of large scale treatment is required, 2) a huge amount of water is necessary and 3) a prolonged period of time is required for the treatment. Secondly, in the process by ultrasonication of the prior art, there are problems in that 1) the throughput of one single treatment is lower, 2) deviation between treating lots is large, since control of sonication efficiency and of sonication time is difficult and monodisperse aggregates are not able to be obtained steadily and 3) probable contamination with, for example, fractured metal fragments may occur~~red~~ due to deterioration of the sonication tip~~ may occur~~.

~~On~~With the background described as above, a large scale preparation of aggregates of a hydrophobic group-containing polysaccharide is difficult by the processes

with dialysis and ultrasonication of the prior art. Therefore, a more simple and convenient process for forming aggregates of a hydrophobic group-containing polysaccharide is expected. However, no technique of forming aggregates of a hydrophobic group-containing polysaccharide has hitherto been known other than the above-mentioned processes of dialysis and ultrasonication.

While a homogenizer is used for emulsifying oils in water, no practical experience has heretofore been known in which a homogenizer is used for forming aggregates of a hydrophobic group-containing polysaccharide.

The object of the present invention is to obviate the problems in the prior art described above and to provide a process for forming aggregates of a hydrophobic group-containing polysaccharide, in which the deviation between treating lots and the contamination by impurities are eliminated and which can afford to prepare uniform aggregates of a hydrophobic group-containing polysaccharide steadily in a simple and convenient way within a brief time and in a large scale.

DISCLOSURE OF THE INVENTION

The inventors reached from their sound researches ~~a~~the knowledge that aggregates of a hydrophobic group-containing polysaccharide can be obtained in a simple and convenient way within a brief time in large scale by dispersing a swollen liquor of the hydrophobic group-containing polysaccharide using a high pressure homogenizer, whereby the present invention has been completed. Thus, the present invention consists in the process for forming aggregates of a hydrophobic group-containing polysaccharide as given below:

(1) A process for forming aggregates of a hydrophobic group-containing polysaccharide in water, comprising

Please replace the BRIEF DESCRIPTION OF THE DRAWINGS section, beginning on page 8, line 4, with the following rewritten section.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the results of Example 1-1 in graphs, each in a chart of the results of SEC analyses of a pullulan-cholesterol derivative (CHP) before and after the treatment by a high pressure homogenizer. Figs. 1(a) and 1(b) are each a chart of analysis results of SEC before and after the treatment of the CHP by the high pressure homogenizer, respectively. The ordinate represents the strength (dimensionless) of the differential refractometer (the same applies to those in the following).

Fig. 2 shows the results of Examples 1-2 to 1-5 in graphs, wherein Figs. 2(a), 2(b), 2(c) and 2(d) are each a chart of analytical results of SEC after the treatment by high pressure homogenizer for Examples 1-2, 1-3, 1-4 and 1-5, respectively.

Fig. 3 shows the results of Comparative Example 1 in a graph, a chart of the results of SEC analysis after the dialysis.

Fig. 4 shows ~~by~~charts of the results of SEC analyses of pullulan (of a molecular weight of 108,000) and of CHP. Figs. 4(a), 4(b) and 4(c) are each a chart of the results of SEC analysis, for the pullulan (molecular weight of 108,000), for the CHP and for the aggregates of the CHP, respectively.

Fig. 5 is a chart of the results of SEC analysis of CHP (a concentration of 0.2% by weight) after ~~an~~ultrasonication for a predetermined period of time.

Fig. 6 shows the results of Comparative Example 2 in graphs, namely, charts of the SEC analysis results of a CHP after an ultrasonication treatment and after a treatment by a high-pressure homogenizer, respectively. Figs. 6(a) is a chart of the results of SEC analysis of the CHP after the ultrasonication treatment. Fig. 6(b) is a chart of the results of SEC analysis of the ultrasonicated liquor of Fig. 6(a) after it is treated by the high-pressure homogenizer.

Please replace the paragraph beginning on page 9, line 15, and ending on page 9, line 27, with the following rewritten paragraph.

While there is no special limitation for the hydrophobic group-containing polysaccharide to be employed according to the present invention, so long as it has hydrophobic groups, the following hydrophobic group-containing polysaccharides are preferred. Thus, preference is given to polysaccharides having -XH groups (wherein X denotes an oxygen atom or a nitrogen-containing group represented by NY, with Y standing for a hydrogen atom or a hydrocarbon group of 1 - 10 carbon atoms), wherein 0.1 - 10, preferably 0.1 - 6, -XH groups per 100 monosaccharide units constituting the polysaccharide are replaced by one or more hydrophobic groups represented by the formula (1) given above.

Please replace the paragraphs beginning on page 14, line 9, and ending on page 19, line 21, with the following rewritten paragraphs.

The amount of water to be used in the process step [1] may favorably be 30 - 10,000 times by weight, preferably 100 - 1,000 times by weight, of the hydrophobic group-containing polysaccharide. If this amount is short of 30 times by weight, the hydrophobic group-containing polysaccharide may become an unfavorabley gelled state. If this amount exceeds over 10,000 times by weight, the efficiency of forming aggregates will become unfavorably decreased. While there is no special restriction as to the water temperature for effecting swelling, a temperature of 0 - 100 °C, preferably 10 - 50 °C, may be favorable.

The resulting swollen dispersion may favorably be brought to the subsequent process step [2] after having been stirred by a stirrer. As the stirrer to be employed, a magnetic stirrer, a homomixer or the like may be exemplified. Among them, preference is given to the homomixer. While there is no special limitation for the revolution rate, stirring duration and so on of the stirrer, a revolution rate of 100 - 15,000 rpm and a stirring duration of 30 seconds to 180 minutes may be favorable. The dispersion resulting from stirring of the swollen dispersion is present as a turbid liquid, which gives birth to deposition of precipitates after standing for a while.

The homogenizer to be employed in the process step [2] should be capable of dispersion-treating the swollen dispersion from the process step [1] under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2), preferably 98 - 294

MPa (1,000 - 3,000 kgf/cm^2). For such a homogenizer, a commercial high pressure homogenizer may be employed. A high pressure homogenizer is a device for attaining emulsification of microdispersion of a liquor by generating shearing forces, impingement momentums and cavitation by the aid of a high pressure.

When such a high pressure homogenizer is used, aggregates of the hydrophobic group-containing polysaccharide can be formed, ~~concretely~~specifically, in the following manner. First, the swollen dispersion is pressurized at a pressure mentioned above and the so-pressurized swollen dispersion is spouted from an orifice into a chamber to cause cavitation (pressure drop). The spouted swollen dispersion is thereby accelerated and ~~is~~caused to bring about intense collisions of domains of the swollen dispersion with each other in the chamber and with the walls of the chamber. By the thereby generated impingement momentums and shearing forces, the hydrophobic group-containing polysaccharide is dispersed finely in the dispersion to build up aggregates thereof. The so-obtained treated liquor is present as a transparent colorless liquid which is a dispersion (expressed in the following sometimes as aqueous solution) not subject to the occurrence of turbidity or precipitation after a prolonged standing ~~still~~period.

The dispersing treatment using a high pressure homogenizer may be effected only once or in two or more ~~repeats~~repetitions. The treatment with high pressure homogenizer may be carried out in a batchwise or continuous operation. While the number of ~~repeats~~repetitions of the high pressure homogenizer treatment may vary considerably depending on, for example, each specific hydrophobic group-containing

polysaccharide, the degree of substitution with ~~such~~the hydrophobic group, the concentration in the aqueous dispersion and the pressure ~~on~~of the high pressure homogenizer treatment, a stable and relatively monodisperse aggregate may be obtained usually by five ~~repeats~~repetitions, though not affirmable. For example, in the case where the hydrophobic group-containing polysaccharide is a pullulan-cholesterol derivative with a cholesterol-substitution degree of 1.2 cholesterol groups per 100 monosaccharide units, the concentration in the aqueous dispersion is 0.2% by weight and the pressure on the high pressure homogenizer treatment is 98 MPa (1,000 kgf/cm^2), a stable aggregate without suffering from the occurrence of turbidity or precipitation can be obtained by repeating the dispersing treatment by the high pressure homogenizer three times.

Concrete examples of the high pressure homogenizer which can be used in the process according to the present invention include MICROFLUIDIZER (of the firm Microfluidex, trademark), MICROFLUIDIZER (of Mizuho Kogyo K.K., trademark), DeBEE 2000 (trademark, supplied from Q.P. Corp.) and APV GAULIN (trademark, of APV Rannie, Inc.).

While there is no special limitation as to the temperature of the swollen dispersion ~~on~~during the dispersing treatment by a homogenizer, a temperature in the range from 0 to 100 °C, preferably from 10 to 50 °C, may be favorable.

By performing the dispersing treatment using a homogenizer, a monodisperse aggregate can be formed. The resulting monodisperse aggregate, namely, the aggregate of the hydrophobic group-containing polysaccharide obtained by the process according to the present

invention, has usually an aggregate particle size in the range from 10 to 30 nm and a number of associations of the hydrophobic group-containing polysaccharide in the aggregate in the range from 3 to 20. Here, the particle size and the number of associations refer both to the average value. The resulting treated dispersion is a colorless transparent aqueous solution which will not become turbid nor bring about precipitation after a prolonged standing ~~still~~period. Here, the monodisperse aggregate will not be formed by simply agitating the swollen dispersion by a stirrer, such as a magnetic stirrer or homomixer. The swollen dispersion keeps its turbid state and will not turn into a colorless transparent state even though the revolution rate of the stirrer is increased or the stirring is continued for a prolonged period of time.

The aggregate of the hydrophobic group-containing polysaccharide formed by the process according to the present invention can be separated in a form of a solid matter by drying the aggregate, after it has been formed, by means of, for example, freeze-drying. From this solid matter, a colorless transparent aqueous solution of the aggregate in the state before the freeze-drying can be restored by adding water to the solid matter.

The aggregate of the hydrophobic group-containing polysaccharide formed by the process according to the present invention can be used as a medicinal material, such as a coating material for coating a drug carrier enclosing therein a drug. Thus, it can be used as a coating material for coating a drug carrier made of, for example, a liposome, microcapsule, microsphere, O/W emulsion or erythrocyte ghost. Here, the aggregate of the hydrophobic group-containing polysaccharide obtained

by the process according to the present invention can be used securely as a medicinal material and for preparing a drug carrier of a stable quality, since the so-obtained aggregate is a homogeneous product and has no quality deviation between production lots nor contamination by impurity~~y~~ies. The aggregate of a hydrophobic group-containing polysaccharide obtained by the process according to the present invention can also be utilized as a surfactant~~s~~, thickening agent~~s~~ and a raw material for cosmetics.

In the process according to the present invention, it is possible to employ a mixture of a hydrophobic group-containing polysaccharide with one or more polysaccharides having no hydrophobic group (i.e. those before introduction of hydrophobic group therein) and/or one or more medicaments and/or one or more proteins, instead of using the hydrophobic group-containing polysaccharide solely. Hereby, the possibility of extension of application field, for example, in the drug delivery system (DDS), may be prospective.

As described above, aggregates of a hydrophobic group-containing polysaccharide can securely be formed in a homogeneous quality steadily within a brief period of time, in a large scale and in a simple manner, by the process according to the present invention without suffering from quality deviation between production lots and from contamination by impurity~~y~~ies, since the process is performed by a dispersing treatment of a swollen dispersion of the hydrophobic group-containing polysaccharide using a homogenizer under a pressure within a specific range.

Please replace the paragraph beginning on page 20, line 25, and ending on page 21, line 14, with the following rewritten paragraph.

An eggplant type 1-liter flask was charged with 25 grams (0.065 mol) of cholesterol and thereto were added 300 ml of toluene to dissolve it, whereto 17 ml (0.12 mol) of triethylamine were added. To this, 161 grams (0.96 mole, 14.8 eq.) of hexamethylene diisocyanate dissolved in 300 ml of toluene were added to cause a reaction at 80 °C for 6 hours under a nitrogen atmosphere. After termination of the reaction, toluene and the excess amount of hexamethylene diisocyanate were removed by reducing the pressure. The resulting yellowish oily residue was ~~stood still~~allowed to stand overnight at room temperature to cause precipitation of pale yellow crystals. The crystals were taken out and about one liter of hexane was added thereto, whereupon the mixture was shaken vigorously and, then, the supernatant liquid was removed by decantation. This washing procedure was repeated four times, whereupon the crystals were dried under a reduced pressure at room temperature for three hours, whereby N-(6-isocyanatohexyl)cholesteryl carbamate represented by the following formula (4a) was obtained.

Please replace the paragraph beginning on page 21, line 18, and ending on page 14, line 22, with the following rewritten paragraph.

In an eggplant type flask of 300 ml capacity, there were charged 3.48 g (12.9 mmol) of stearyl alcohol and thereto were added 50 ml of toluene to dissolve it,

whereto 2.04 g (25.8 mmol) of pyridine were further added. To this mixture, there were added 30 g (178 mmol, 14.8 eq.) of hexamethylene diisocyanate dissolved in 50 ml of toluene and the resulting mixture was subjected to reaction at 80 °C under a nitrogen atmosphere for about 3 hours. After termination of the reaction, toluene and the excess ~~of~~hexamethylene diisocyanate were removed under a reduced pressure, whereby ~~a~~pale yellow crystals were formed. The crystals were taken out, whereto about one liter of hexane was added and the mixture was shaken vigorously, whereupon the supernatant was removed by decantation. This washing procedure was repeated four times, whereupon the product was dried under a reduced pressure for three hours at room temperature. Hereby N-(6-isocyanatohexyl)stearyl carbamate represented by the following formula (8) was obtained:

Please replace the paragraphs beginning on page 22, line 19, and ending on page 23, line 30, with the following rewritten paragraphs.

A hydrophobic group-containing polysaccharide was synthesized according to the method of Akiyoshi et al {Macromolecules,. 3062 (1993)}. Thus, an eggplant type flask of 1 liter capacity was charged with 40 g (248 mmol as anhydrous glucose unit) of a pullulan (a product of Wako Pure Chemical Industries, Ltd.; average molecular weight: 108,000) and 420 ml of dimethyl sulfoxide (sometimes abbreviated as DMSO) and the mixture was agitated at 80 °C under a nitrogen atmosphere to dissolve it. To this solution, a solution of 1.78 g (3.21 mmol) of N-(6-isocyanatohexyl)cholesteryl carbamate synthesized in Synthesis Example 1-1 dissolved in 32.4 ml (0.40 mol)

of pyridine was added and the mixture was subjected to reaction at 90 °C for 1.5 hours.

After termi~~a~~nation of the reaction, dimethyl sulfoxide was removed by reducing the pressure and the resulting oily residue was dropped into 6 liters of acetone to form a precipitate which was purified. After removal of the supernatant, 4 liters of acetone were added to the resulting precipitate and the mixture was ~~stood still~~allowed to stand overnight at room temperature. The precipitate was collected by filtration and ~~was~~dried under a reduced pressure. The so-obtained solids were dissolved in dimethyl sulfoxide and the solution was charged in a dialysis bag (Spectra/Por3, a product of the frim Spectropor; a fractionating molecular weight of 3,500) and was subjected to a dialysis against distilled water for one week. 1.5 liters of the resulting polymer solution were treated by freeze-drying in an ordinary manner, whereby a pullulan-cholesterol derivative (abbreviated hereinafter sometimes as CHP) represented by the following formula (7a) was obtained. By calculating the proportion of introduction of the cholesteryl groups into the pullulan in the CHP from the integration value of the ^{1}H-NMR spectrogram of CHP, it was determined that the proportion of substitution with cholesteryl group in the pullulan-cholesterol derivative (CHP) represented by the formula (7a) was about 1.3 groups per 100 monosaccharide units.

Please replace the paragraph beginning on page 24, line 8, and ending on page 24, line 16, with the following rewritten paragraph.

In the same manner as in Synthesis Example 2, except that a commercial mannan (a produ~~a~~ct of the firm Sigma) having an average molecular weight of about 85,000 was used in ~~the~~place of the pullulan, a mannan-cholesterol derivative (in the following, sometimes abbreviated as CHM), in which about 2.3 cholesteryl groups are introduced per 100 monosaccharide units, represented by the following formula (7b) was synthesized.

Please replace the paragraph beginning on page 25, line 14, and ending on page 26, line 19, with the following rewritten paragraph.

There were added 1,000 ml of water to 2 grams of the CHP obtained in Synthesis Example 2 to cause the CHP to swell at a temperature of 60 °C for 2 hours (CHP concentration = 0.2 % by weight). The resulting swollen dispersion was then stirred using a homomixer (5,000 r.p.m.) for 5 minutes. The appearance of the dispersion at this occasion was white turbid. The so-stirred swollen dispersion of 20 ___°C was subjected to a homogenization by causing the dispersion to spout out of an orifice under a pressure of 98 MPa (1,000 kgf/cm^2) using MICROFLUIDIZER (trademark, a high pressure homogenizer Model M-110Y of the firm Mizuho Kogyo K.K.) into a chamber in order to disperse it. This homogenization treatment was repeated twice. The herein used MICROFLUIDIZER had a treating capacity of about 500 ml/min. and the time required for ~~the twice repeats~~two repetitions of the homogenization treatment was about 5 minutes. The resulting treated liquor had a colorless and transparent appearance. For this aqueous solution, the particle size and the number of

associations were determined by the methods indicated above. The results are summarized in Tables 1 and 2.

Please replace the paragraph beginning on page 26, line 29, and ending on page 27, line 12, with the following rewritten paragraph.

Then, the resulting aqueous solution of the CHP aggregates was subjected to a freeze-drying, whereby the aggregates of the CHP were isolated as a white solid matter. To this solid matter, water was added so that a concentration of 0.2 % by weight would be reached, whereupon the mixture was ~~stood still~~allowed to stand at room temperature for three hours in order to restore an aqueous solution. The restored solution was colorless and transparent. For the aqueous solution of the CHP aggregates before the freeze-drying and for the restored solution, SEC analyses were carried out, whereby it was recognized that there was no distinction in the chart curve between both the solutions and ~~was~~confirmed that both ~~are~~were identical.

Please replace the paragraph beginning on page 27, line 23, and ending on page 27, line 30, with the following rewritten paragraph.

By performing the freeze-drying in the same manner as in Example 1-1, the aggregates in each Example were isolated in a form of white solid matter. For the aqueous solution of the aggregates before and after the freeze-drying, comparison was carried out as in Example 1-1, whereby it was recognized that there was no

distinction therebetween and ~~was~~confirmed that both ~~are~~were identical.

Please replace the paragraphs beginning on page 32, line 28, and ending on page 33, line 29, with the following rewritten paragraphs.

From the results given above, it is seen that a sufficient formation of aggregate was not able to be attained using an ultrasonication, whereas the process as shown in the Examples using a high pressure homogenizer was able to attain formation of an aggregate easily.

It is also seen that aggregates exhibiting a narrower molecular weight distribution were formed in Examples 1-1 to 1-6 in which a high pressure homogenizer was used, as compared with the results of Comparative Example 1 in which dialysis was employed and of Comparative Example 2 in which an ultrasonic wave treatment was used. It is further seen that aggregates of a hydrophobic group-containing polysaccharide can be formed within a brief time in a simple and convenient manner in large amount by the process according to the present invention, since inventive Example 1-1 showed a productivity of 2 grams in a treating time of 5 minutes, whereas Comparative Example 1 using dialysis showed a productivity of 2 grams in a treating time of 4 days and Comparative Example 2 using ultrasonication showed a productivity of 2 grams in a treating time of more than two hours.

INDUSTRIAL APPLICABILITY

The aggregates of a hydrophobic group-containing polysaccharide formed by the process according to the

present invention can be utilized as a coating material for coating drug carriers encapsulating therein drugs. For example, it can be used as the coating material for coating drug carriers, such as liposome microcapsules, microspheres, O/W emulsions and erythrocyte ghosts.

IN THE ABSTRACT

Please amend the abstract as follows.

PROCESS FOR FORMING AGGREGATES OF HYDROPHOBIC GROUP-CONTAINING POLYSACCHARIDE

ABSTRACT

A process for forming aggregates of a hydrophobic group-containing polysaccharide in water, which ~~comprises~~involves the steps of causing the hydrophobic group-containing polysaccharide to swell in water and treating the resulting swollen dispersion by dispersing it using a homogenizer under a pressure of 9/8 - 490 MPa (100 - 5,000 kgf/cm^2), whereby homogenous aggregates of the hydrophobic group-containing polysaccharide are formed in a simple and convenient way in a large amount and within a brief time.